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HtpG and *STM4067* contribute to long-term *Salmonella* Typhimurium persistence in pigs

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Introduction

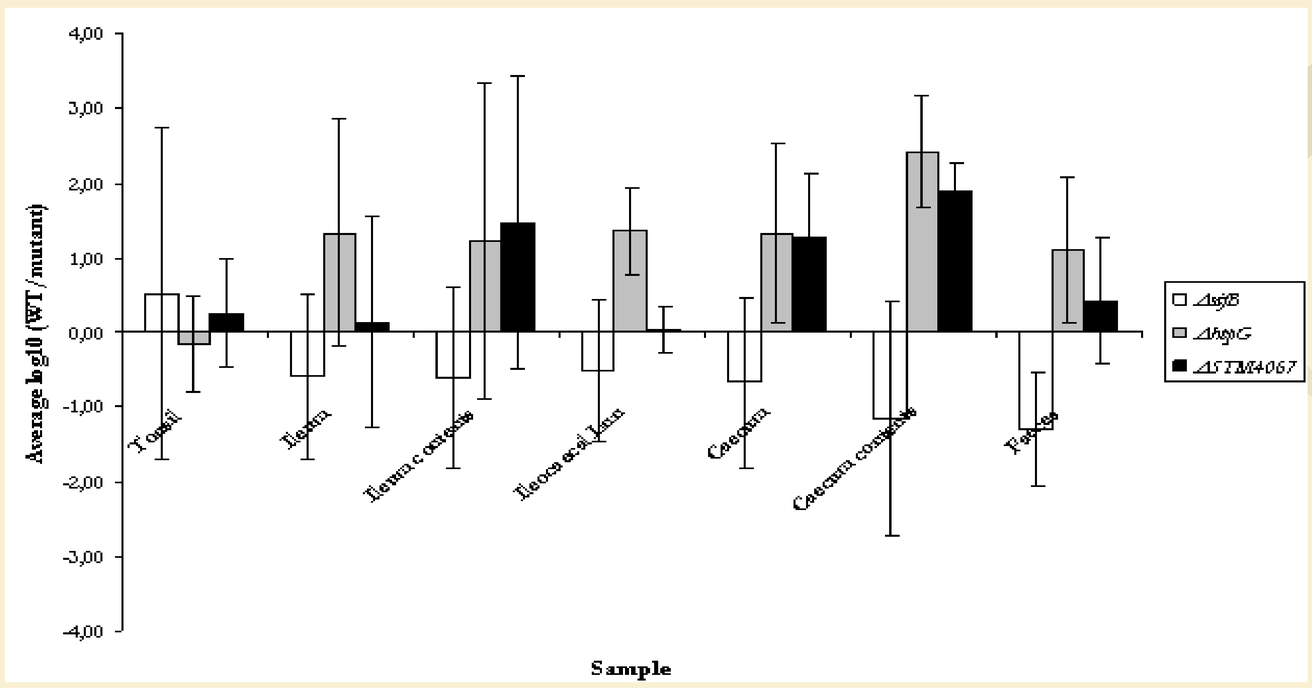
Persistent *Salmonella* Typhimurium infections in pigs often result in asymptomatic carrier pigs and are a major concern for food safety and human health. Tonsils and lymph nodes play a key role in the persistence of *Salmonella* Typhimurium in pigs, but very little is known about the underlying mechanisms. After bacterial invasion in pigs, the porcine immune system will respond to clear the *Salmonella* infection and bacterial survival strategies for (long-)term persistence will become important. For the identification of *Salmonella* Typhimurium genes specifically induced in tonsils and lymph nodes at 3 weeks post inoculation, a genome-wide screening method was performed using *in vivo* expression technology (IVET)[1].

Materials and Methods

We used a spontaneous nalidixic acid resistant derivative of a virulent wild type *Salmonella* Typhimurium (WT) strain isolated from a pig stool sample. For proper use in the IVET protocol, we verified if a *purA Salmonella* Typhimurium mutant[2] was significantly impaired in comparison to the wild type strain in an *in vivo* mixed infection experiment. We constructed an IVET library as described earlier for *Salmonella* Enteritidis[3], composed of approximately 12,000 different transformants, representing the major part of the *Salmonella* Typhimurium genome.

For IVET selection in pigs, 9 pigs were orally inoculated with 10^8 colony forming units (CFU) of the IVET library. Three weeks after inoculation, pigs were euthanized and tonsils and lymph nodes were collected, processed and plated on selective MacConkey agar. White colonies, representing transformants with *in vivo* but not *in vitro* induced genes, were collected and the IVET fusions were sequenced as described before[3]. Identification of the sequence of the cloned promoter was done by BLAST analysis.

Three mutants in *in vivo* induced genes were constructed[2] (Δ*sifB*, Δ*htpG* and Δ*STM4067*) and tested in a subsequent mixed infection experiment. Three groups of 6 piglets were inoculated with 2 x 10^7 CFU of the WT and 2 x 10^7 CFU of 1 of the 3 constructed mutants. After euthanization, tonsils, ileum (+contents), ileocaecal lymph nodes, caecum (+contents) and faeces were analyzed for the number of *Salmonella* Typhimurium bacteria.



▲**Figure 1.** Recovery of bacteria from various organs of six piglets orally inoculated with a mixture of the wild type *Salmonella* Typhimurium and Δ*sifB*, Δ*htpG* and Δ*STM4067 Salmonella* Typhimurium. The average log10 value of the ratio of CFU per gram sample of the wild type and the mutants is given ± SD.

Gene family	Tonsils	Ileocaecal lymph nodes
Virulence	***sifB***	***sifB***
Stress	***htpG***	***htpG*** *dnaK*
Motility	*fliC/fljB*	
LPS		*rfaE*
Metabolic	*efp* *dnaC/dnaT* *gyrB* *rnt* *pflC* *rpsU* *artP* *kdgK* *lysS*	*efp* *dnaC/dnaT* *gyrB* *rnt* *pflC* *aroK* *pflD* *fnr* *rpoZ* *nrdB* *yhbG/rpoN* *ssb/asmA*
Unknown	*ygfA* *ybjP* ***STM4067*** *ydeW* *yggE*	*ygfA* *citX2/citG2* *yaeT* *yadF*

▲**Table I.** Overview of *in vivo* induced *Salmonella* Typhimurium genes in tonsils and lymph nodes at 3 weeks post inoculation of pigs, identified by *in vivo* expression technology. The genes that were tested for their role in long-term *Salmonella* persistence in pigs in a subsequent mixed infection experiment, are marked in bold.

Results

From the IVET selection experiment, 19 and 24 *in vivo* expressed genes were identified in the tonsils and lymph nodes respectively (summarized in Table I). One known virulence gene (*sifB*) and 2 genes encoding factors playing a role in *Salmonella* stress responses (*htpG* and *dnaK*) were identified. The majority of the identified genes plays a role in *Salmonella* metabolism or exert a yet unknown function in *Salmonella* persistence in pigs. From the 3 genes examined in the subsequent mixed infection experiment, Δ*htpG* and Δ*STM4067* were impaired in colonization of the intestines and lymph nodes of pigs, compared to the wild type; this was not the case for Δ*sifB*. Furthermore, none of the 3 examined *Salmonella* genes played a role in *Salmonella* persistence of porcine tonsils (Figure 1).

Discussion

Using IVET, Huang *et al.* already identified several *Salmonella* Typhimurium genes expressed in porcine tonsils at 2 days post inoculation[4]. These genes differ from the genes that we were able to identify at 3 weeks post inoculation (except for *rpoN*), suggesting that different sets of *Salmonella* genes play a role in short- and long-term persistence in pigs. Furthermore, we were able to show a role for *Salmonella* Typhimurium genes *htpG* (encoding a heat-shock protein) and *STM4067* (encoding a protein with an unknown function) in long-term persistence in the porcine intestines and lymph nodes, although their exact role remains to be clarified.

References

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